ON THE MORPHOLOGY OF THE NEPHRIDIA OF NEREIS LIMNICOLA JOHNSON

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In the past century there have been a number of reports of observations on the nephridia of the polychaetous annelids. These have ranged from passing notes to detailed morphological treatments. Some of these have been concerned with all families of the polychaetes or with general comments (Benham, 1891; Ehlers, 1864-68; and Goodrich, 1895, 1945); some dealt only with the nephridia of the so-called errant forms (Aiyar, 1933; Fage, 1906; and Goodrich, 1897, 1898 and 1900); and most have considered the nephridia of the so-called sedentary worms.

Relatively little information has been published on the detailed morphology of the nephridia of the Nereidae. Goodrich (1893) described the nephridia of *Nereis diversicolor* and found that the nephridial canal could be divided into four regions which differed in distribution of cilia, diameter of the lumen and extent of tubule convolution. Fage (1906) worked on *Perinereis cultrifera*, observing nephridial histology and reporting on the uptake of neutral red by nephridia in living animals. Krishnan (1952) described the nephridial morphology of three nereid species with contrasting salinity tolerances: *Namalycastis indica* (Southern) (euryhaline); *Nereis chilkaensis* Southern (relatively stenohaline, in slightly brackish environments); and *Perinereis nuntia* (Savigny) (stenohaline, in fully marine situations). Krishnan not only described the nephridia of the three genera, but also related the size of the nephridia and the amount of nephridial vascularization to the ability of the polychaetes to tolerate lowered salinities. He concluded that the large size of the nephridia of *N. indica*, as well as their rich supply of blood vessels, represents an adaptation for a euryhaline existence. He suggested that there is a direct excretion of water from blood vessels to the lumen of the nephridial canal and showed that there is a shrinking and collapse of nephridial blood vessels in specimens of *N. indica* which had been acclimatized to full-strength sea water. Finally, the nephridial morphology of *Nereis vexillosa* Grube has been described (Jones, 1957) and it was noted that the nephridial canal of this species is ciliated throughout and that there are three general regions along the length of the canal, based on the lumen diameter [1] and the amount of convolution. In addition, a reconstruction of the nephridial canal was presented.

Nereis limnicola Johnson has recently been used as an experimental animal by a number of workers. The species was originally described (Johnson, 1903) from Lake Merced, a fresh-water lake which has served as a water supply for the city of

[1] It should be noted that in Figure 8, Jones, 1957, the scale line of the diagram of the nephridial canal of *Nereis vexillosa* should read 100 micra, not 50; further, Figure 6 is reversed, left for right.

San Francisco, California (for a short account of the history of the lake, see Smith, 1958, p. 61). Subsequently, there were no published reports of the species until Smith (1958) re-collected material from Lake Merced for physiological observations.

Hartman (1938) described and recorded (1944) *Neanthes lighti* from Marin and Sonoma Counties, north of San Francisco Bay. Smith (1950) described embryonic development in specimens of this species from the Salinas River, south of San Francisco, near Monterey, and showed that it is a viviparous self-fertilizing hermaphrodite. Later, Smith (1953) studied the distribution of the species along the Salinas River and reported observations on the salinity cycle of the river over a three-year period and the effect of salinity changes on the distribution of the polychaete.

After his re-collection of *Nereis limnicola,* Smith (1959b) compared the type specimens of *Nereis limnicola* Johnson (1903) with specimens of *Neanthes lighti* Hartman (1938) and concluded that *Neanthes lighti* is a junior synonym of *Nereis limnicola. Neanthes lighti* was referred to *Nereis japonica* Izuka (1908) by Edith and Cyril Berkeley (1956, p. 269), who pointed out the close morphological similarities between *Nereis japonica* and *Nereis diversicolor* O. F. Müller. Smith (1958) compared, in considerable detail, specimens of *Nereis limnicola* from California, Washington, and British Columbia, with specimens of *Nereis japonica* from Japan, and specimens of *Nereis diversicolor* from Scotland, England, Denmark, Finland, France, and New Hampshire. Smith presented strong arguments for the separation of these three species, which are reproductively and geographically isolated. Their close morphological and ecological similarities are emphasized by Pettibone (1963, pp. 160–161) who referred all three species to *Nereis (Hediste).* Hartman (1960) referred *N. limnicola, N. lighti* and *N. japonica* to *Neanthes diversicolor* and Imajima and Hartman (1964) referred *Nereis japonica* Izuka to *Neanthes diversicolor.* However, I prefer to follow Smith (1958, 1959b), Khlebovich (1963), and Pettibone (1963) in considering the three species, *N. diversicolor* O. F. Müller, *N. japonica* Izuku, and *N. limnicola* Johnson, as distinct, but closely related, species of *Nereis (Hediste).*

Nereis limnicola has been utilized by Smith for physiological studies (1957, 1959a), who found that the species can control the influx of pond water, distilled water, and extreme dilutions of sea water at 13° C., but has no control at temperatures of 1°–2° C. Later, Smith (1963), in comparing *N. diversicolor, N. limnicola,* and *Nereis (Neanthes) succinea,* found that *N. limnicola* had the lowest salt loss rate of the three species when placed in lowered salinities, but (Smith, 1964) that both *N. limnicola* and *N. succinea* have an equal D_2O influx at a body weight of about 100 mg., even though *N. limnicola* takes up less water when both species are subjected to an equal external osmotic gradient.

Stephens (1964) made observations of the uptake of glycine by *N. limnicola* and *N. succinea.* He found that the uptake by the latter is greater by an order of magnitude than in the former and suggested that the uptake takes place across the body wall. Stephens further suggested that glycine uptake and osmoregulation are incompatible, since glycine uptake becomes less, and even ceases, when the salinity of the medium is lowered into the range wherein the worms are hyper-regulating.

Oglesby (1965a), in comparing water and chloride regulation in *N. limnicola,*

N. succinea, *N. vexillosa*, and *Laconereis culveri* (Webster), reported that *N. limnicola* shows the best ability to regulate osmotic concentration and exhibits the least change in water content of the entire body with varying salinities. Further, Oglesby (1965b) has shown that the chloride exchange rate is lowest in *N. limnicola* and suggested that this may be due to a low chloride permeability, the worms becoming essentially impermeable to chloride in fresh water.

In his paper treating of viviparity in *Nereis limnicola*, Smith (1950) dealt with worms inhabiting the lower Salinas River, Monterey County, in central California. The Salinas River presents a difficult situation for aquatic forms. It is not a large river and appears to serve mainly as a run-off channel for the fall, winter, and spring rains from its watershed. After the spring run-off, a sandbar is formed across the mouth of the river, forming a "blind" estuary of the river, as defined by Day (1951). This serves to dam the river flow until the following winter when the press of run-off may be sufficient to break through to the sea. The latter is not necessarily an annual event and the river mouth may remain blocked for several years.

Smith's studies (1950) were concerned with the worms found in two areas. One ("Area A" on Smith's map, p. 425) was a "muddy channel in a *Salicornia* marsh near the mouth of the Salinas River," and the other (Area B) was "a sandy stretch of river about four miles upstream, where the general aspect is that of fresh water." Smith further gave the range of salinities for these areas as 20% to 115% sea water for Area A and 1% to 3% sea water for Area B, the salinity of a given area being a function of rainfall and season. He pointed out that the effect of the more extreme low salinities was possibly damped by the residual salt in the soil of the surrounding substrate. Smith (1953) later provided more detailed ecological observations on the lower Salinas River and referred to seven numbered locations (location 3 = Area A, above; location 4 = Area B).

During the rainy season and the period of run-off, the *Salicornia* marshes of the lower Salinas River may be flooded with fresh water. Within a few days the salinity of the overlying water is decreased immensely. This dilution prevails for a variable period until the last of the seasonal excess is sluiced into the sea. At this time, as the fresh water recedes, the marshes may be inundated with sea water at spring tides. As the flow of the river decreases, the channels in the marsh become isolated from the main river. Through the summer, isolated ponds are subjected to the evaporative effect of the sun, although they are relieved by sporadic rains and heavy fogs. During this time, the salinity of the overlying water reaches its annual high that is maintained until the fall rains, when the rising river inundates the isolated areas and dilutes them to their annual low. In the descriptions and discussion to follow, the worms of Area A will be referred to as the down-river population.

Throughout the year there is near-fresh water over Area B, several miles up-river from Area A. This condition is relieved only during times of extremely high tides when the river flow is slackening, following the winter rains, at which times incursions of salt water may reach Area B. The worms found in this area are probably near the extreme fresh-water end of their range, for Smith (1953, location 5), has not found them more than ½ mile up-stream from the collection site in Area B. The worms of Area B will be referred to as the up-river population.

Considering the brief ecological description above, several questions become apparent. Is it reasonable to expect that groups within the same species, differing only in the salinity of their environments, will exhibit morphological differences? Or, because of a physiological adaptability, will these groups show no significant anatomical differences? If there are differences, will they be manifest in the nephridium? It was in an attempt to answer these questions that this morphological comparison of the nephridia of *Nereis limnicola* from environments of different salinity was undertaken.

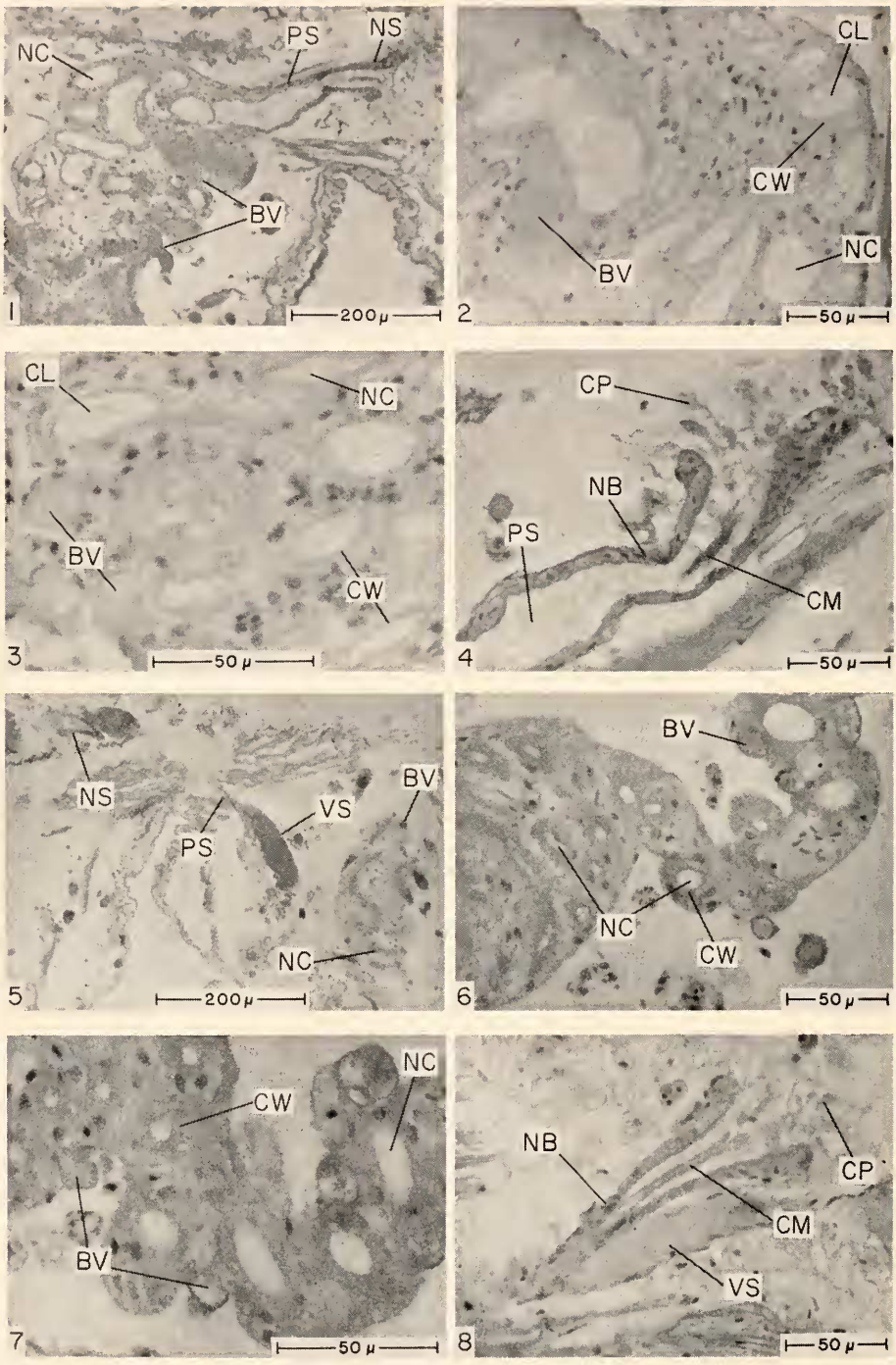
The Nephridium of Nereis limnicola (Up-River Form)

The worms used in the following section were collected in Area B (Smith, 1950). The salinities of the water flowing over them were less than 2.5% sea water. The worms were relaxed by the use of dilute alcohol, fixed in Bouin's, serially sectioned in paraffin at 6 micra, stained in Harris' hematoxylin, and counterstained in eosin.

Within the same animal, indeed, within the same segment, there may be a wide variation in the overall size of the nephridia. In one case, a pair of nephridia in the same segment were observed in which the sizes differed in the order of 1:2. These approximated the extreme differences between pairs of nephridia, as well as between unpaired nephridia. They were both approximately the same width and length (250 micra), but differed, however, in that one was about 240 micra in height while the other was about 400 micra (for lengths and numbers of segments of the worms examined, see Table II). The approximate volume of the former was 0.0106 mm.^{3} and the latter, 0.0140 mm.^{3}

If one views the nephridium of *Nereis limnicola* from Area B, with the purpose of comparing it to that of *Nereis vexillosa* (Jones, 1957), one is immediately impressed by the vast number of blood vessels in contact with, and buried in, the tissue of the nephridium (Figs. 1, 2, and 3, BV). The shape of the nephridium also contrasts with that of *N. vexillosa*. Whereas the nephridium of *N. vexillosa* is globular and possesses a smooth, even surface, that of *N. limnicola* shows a suggestion of a division into regions. The dorsal half of the nephridium is oval in cross-section, and is somewhat compressed antero-posteriorly. The ventral half is nearly circular in cross-section, and is more or less hemispherical. At the equator of the hemisphere, the post-septal canal enters the nephridial mass in company with the ventral segmental vessel, that ramifies over the surface of the nephridium (Fig. 1, PS). At the point of entry of the post-septal canal there is a slight swelling. The surface of the nephridium shows a slight indication of the internal canal in the more vascularized portion, while the other half of the nephridium externally shows a well-defined canal.

The sectioned nephridium of the up-river form of *Nereis limnicola* shows much the same aspect as *Nereis vexillosa* (Figs. 1, 2, and 3), and a number of nuclei are scattered throughout the sectioned area. Further, there are occasional areas of vacuolation, but not to the extent of those observed in *N. vexillosa*. On the whole, the perforations or sections of tubule lumen observed in the sections of the nephridia of the up-river form of *N. limnicola* presented the same appearance as those of *N. vexillosa*.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8

The ciliation of the nephridial canal (Figs. 2 and 3, CL), as noted in this form, did not seem to differ significantly from the pattern seen in *N. vexillosa*. Cilia were noted throughout the length of the canal, from the nephrostome to within 40 to 50 micra of the nephridiopore. As before, there seemed to be no distinct division in the nephridial canal on the basis of its ciliation.

As mentioned above, the walls of the tubules are only occasionally distinct. When present, they consist of vacuolated areas around the periphery of the perforation. They give the appearance of a clear ring around the lumen, and may have some intradivision in the form of faint, thin walls. In the "non-walled" perforation, the fine network of the interstitial tissue comes up to the canal boundary, and no basement membrane is visible. In all probability, there actually is or was a wall present, but staining and/or fixation techniques may not have been adequate to bring it out. A variation of this last type of wall occurs when the area immediately surrounding the perforation appears to be more heavily stained than the adjacent interstitial tissue (Figs. 2 and 3, CW). By careful examination, it is seen that this darkening is due to the presence of a more concentrated net system and many granular inclusions.

In the nephridium of *Nereis vexillosa* it was noted that blood vessels were at a minimum, approaching and possibly contacting the nephridial system at only two points. In *Nereis limnicola* from the up-river area, it is readily seen that the nephridium is penetrated throughout by many vessels. In the main, they are confined to the more peripheral areas, but many branches pass through the center of the mass (Figs. 1, 2 and 3, BV). The ventral segmental vessel, after it approaches the nephridial mass in company with the post-septal canal, ramifies over the lateral face of the nephridium and at several points passes dorsally into the interior. The ventral portion of the nephridium has no internal blood vessels, while the dorsal half contains more vessels than it carries on its surface. Occasionally, there are blood vessels on the surface of the nephridial mass which seem to have sunken into the tissue. They are not surrounded by nephridial tissue, but are in close contact with it over about 180° to 200° of their circumference in section.

In contrast to the long post-septal canal of *Nereis vexillosa*, this structure in the up-river form of *Nereis limnicola* is extremely short (Fig. 1, PS, and Fig. 9A), the length of the former being 250 micra, and that of the latter about 175 micra.

Key to lettering: BV, blood vessel; CL, cilia; CP, cytoplasmic processes of nephrostome; CM, mass of cilia; CW, nephridial canal wall; NB, band of nuclei of nephrostome (= septal band); NC, nephridial canal; NS, nephrostome; PS, post-septal canal; VS, ventral segmental vessel.

Figures 1–4, up-river form of *Nereis limnicola*; Figures 5–8, down-river form of *N. limnicola*.

FIGURE 1. Dorsal view of right nephridium and associated nephrostome; specimen RB.

FIGURE 2. View of nephridial tissue; specimen S-2.

FIGURE 3. Detailed view of nephridial tissue and associated blood vessels; specimen S-2.

FIGURE 4. Nephrostome; specimen RB.

FIGURE 5. Dorsal view of left nephridium; specimen SB.

FIGURE 6. View of nephridial tissue at junction of medial (left) and lateral (right) regions; specimen S-3.

FIGURE 7. Detailed view of nephridial tissue and associated blood vessels; specimen S-3.

FIGURE 8. Nephrostome; specimen S-3.

Further, the diameter of this portion of the nephridial canal is slightly larger in the up-river form of *N. limnicola*. Proceeding through the post-septal canal from the nephridial mass toward the nephrostome, the cross-sections of the isolated canal show the same structure noted in *N. vexillosa*. The wall appears to be vacuolated, with occasional larger nuclei. Through the proximal portion of the post-septal canal, the nuclei are well-scattered along the proximal portion of the post-septal canal, but become more concentrated toward the middle portion, where there are about twelve visible in each section (6 micra thick). Throughout this part of the canal, cilia are visible, distributed around the inner boundary of the wall. The diameter of the lumen is about 15 micra, basally, near the nephridial mass, and distally narrows to about 7 micra. These conditions prevail throughout the proximal 100 micra of the post-septal canal. Where the lumen is at its narrowest, immediately distal to the region just described, and at about the level of the passage of the post-septal canal through the septum, the nuclei within the walls are quite concentrated; 28 to 30 are distributed fairly evenly around the lumen in a section. This concentration of nuclei, in what might be called a "septal band" (Fig. 4, NB), is not so great nor so extensive as in *N. vexillosa*; a comparison shows a more restricted area in *N. limnicola*.

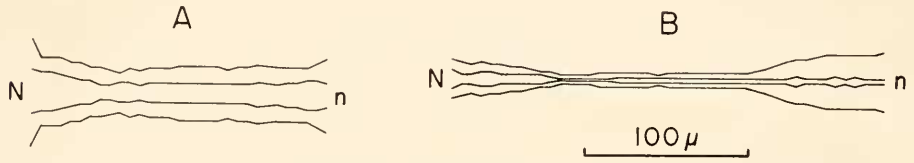


FIGURE 9. Graphic representation of post-septal canals associated with nephridia of *Nereis limnicola*. A. Up-river form; specimen RB. B. Down-river form; specimen SB. N, nephrostome; n, nephridial mass.

Immediately anterior to the narrowing of the canal and the nuclear concentration, the canal widens to form the nephrostome, its extreme width being 25 to 30 micra at its mouth. All along the walls of the funnel the nuclei are scattered evenly, as they were at the beginning of the post-septal canal. As in *N. vexillosa*, the lumen of the nephrostome is almost choked by the tangle of cilia lining it (Fig. 4, CM). Many fairly large club-like structures, the protoplasmic processes, occur at the opening of the nephrostome (Fig. 4, CP). In some of these are found the same type of inclusion that appears in the interstitial tissue and tubule walls of the nephridial mass. It is difficult to make out the exact structure of the processes, for they stain weakly, and, at times, are intermeshed with the cilia that originate in the walls of the nephrostome and the bases of the processes. From the tops and sides of most of the processes, long cilia project into the open mouth of the funnel. It is to be noted that the processes in this species are different from those found in *N. vexillosa*; in *N. vexillosa*, they are long and thin, while in *N. limnicola*, they are stout and nearly pyriform.

Figure 10 shows the diameter of the canal of the up-river form of *Nereis limnicola* from the entrance of the post-septal canal into the nephridium, to the nephridiopore (the canal chosen was the largest of the nephridia observed in detail).

The canal has an overall length of 2232 micra and a mean diameter of 18.9 micra (the length of the canal of the smallest nephridium was found to be 1800 micra and its mean diameter was 19.6 micra). There appear to be four different regions with respect to lumen diameter. The first, diameter about 24 to 30 micra, extends from the entrance of the post-septal canal for about 800 micra; the second, whose diameter is in the range of 36 to 40 micra, extends for another 600 micra; the third, the narrowest part, about 12 to 27 micra, runs for approximately 200 micra before grading into the final portion; the last portion, about 750 micra in length, increases from 27 to 48 micra, then decreases somewhat irregularly until it reaches the nephridiopore, where its diameter is 6 micra. The sudden widening of the canal just prior to the nephridiopore gives the appearance of an ampulla.

In the last portion of the nephridial canal, as the nephridiopore is approached, the wall of the lumen seems to become thicker and more dense. Closer inspection shows that the network of the interstitial tissue has become more concentrated in the immediate area of the canal and that there seems to be an increase in the number

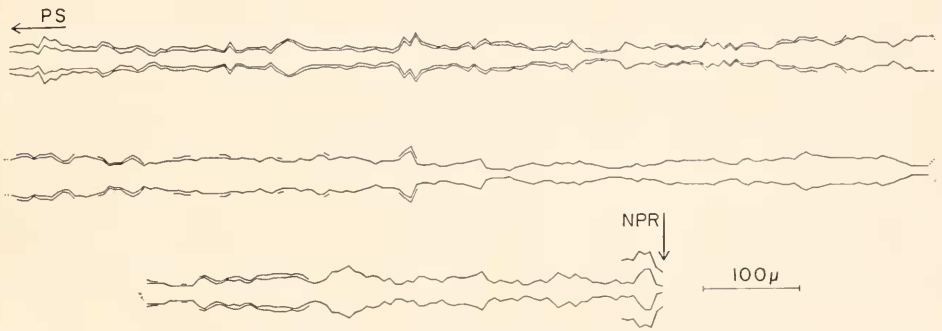


FIGURE 10. Graphic representation of the inner diameter and wall thickness of a nephridial canal of *Nereis limnicola* from up-river (reconstructed from sectioned material). PSC, post-septal canal; NPR, nephridiopore.

of granular inclusions contained in this net. There are large nuclei scattered through the wall of the canal, until, at a point about 40 micra from the external opening, a more regular distribution is assumed, with three or four nuclei apparently in the same plane. This continues to the last 8 micra, where large nuclei are clustered around the canal, and the wall loses its identity in the surrounding tissue. As was the case in *N. vexillosa,* there is no ciliation in the canal through the last 40 to 50 micra. Though an ampulla was not so obvious in *N. vexillosa, N. limnicola* usually shows an ampulla (Fig. 10), or a suppression of one, just interior to the nephridiopore. In cases of suppression, the lumen as seen in section is tripartite, with the walls pressed together until the lumen cross-sectional area is at a minimum. Whether this is an artifact of fixation, a morphological anomaly, a sphincter-like device for closing the canal, or an adaptation providing a greater surface-volume ratio for more efficient resorption or excretion, is not clear, but, as this type of structure was fairly common, the condition seems most probably related to resorption-excretion or to canal closure.

The Nephridium of Nereis limnicola (Down-River Form)

The worms used as a basis for the following description were collected at Area A (Smith, 1950). The salinity of the water standing over them was at least 47.5% sea water. (It is necessary to point out that one of the worms, S-3, was obtained from Dr. Ralph I. Smith, who had adapted it from 81% sea water to 106%. It is assumed that consideration of this worm is not remiss, for this salinity is well within the range reported for the species and at none of these salinities are the worms osmoregulating.) As before, the worms were relaxed, fixed in Bouin's, sectioned serially at 6 micra, stained in Harris' hematoxylin, and counterstained with eosin.

One of the most obvious characteristics of the nephridia of the down-river form of *Nereis limnicola* is their shape. Whereas in the up-river form there was a

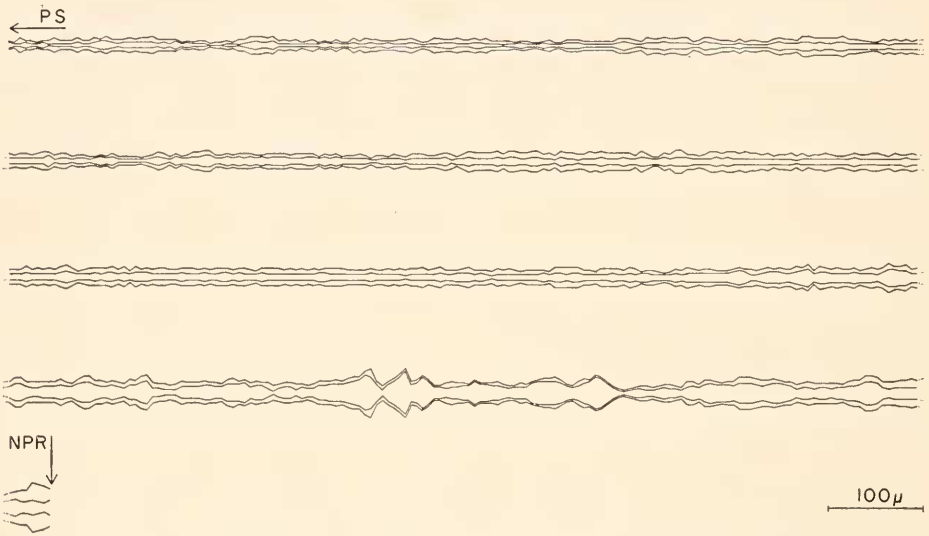


Figure 11. Graphic representation of the inner diameter and wall thickness of a nephridial canal of *Nereis limnicola* from down-river (reconstructed from sectioned material). PSC, post-septal canal; NPR, nephridiopore.

slight compression in the dorsal portion and the ventral half was hemispherical, in the worms from the *Salicornia* marsh, there is a general compression of the entire nephridium (Fig. 5). This usually is seen to occur parallel to the axis of the post-septal canal which projects obliquely, anteriad and mediad. In extreme dimensions, the nephridium measures about 400 micra long, 350 micra high, and 150 micra thick (through the medial half). The lateral half is approximately 50 micra thick, and the approximate volume is 0.0122 mm.^{3}

As just intimated, there is an external division into medial and lateral halves, the medial half being elliptical in cross-section, and the lateral half being extremely compressed to about one-third the thickness of the other. In the extreme dorsal sections of the nephridium the two halves are entirely separate.

In comparing the sectioned nephridia of the down-river and the up-river forms of *N. limnicola,* the first glance at those from down-river would lead one to doubt

that the two were at all related. A considerable reduction occurs in the diameters of the canal lumen, and, to a lesser degree, a reduction in the number of blood vessels (Fig. 5). In addition, the interstitial tissue seems more dense than that in either the up-river form or in *N. vexillosa*.

The diameter of the tubule lumen is, in most portions, as little as 1 to 2 micra. In some cases, the lumen of the canal is almost completely closed and only a pinpoint of clear area is visible by careful focusing. Under these circumstances no measurement is possible, and in graphing the tubule diameter (Fig. 11), these perforations were considered to be one micron or less in diameter. At other times, the perforations were obscured either by heavy ciliation or a turning of the canal within the 6-micron thickness of the section. It is possible that this general narrowness of the canal might have been an artifact caused by osmotic factors during fixation and/or relaxation. However, there were portions of the tubule present in the same section, with diameters comparable to those of *N. vexillosa* and the up-river form of *N. limnicola* (Figs. 6 and 7). In some, a well-defined boundary was visible, complete with a basement membrane separating the tubule wall from the interstitial tissue. In others, there was an irregular area of vacuolation surrounding the perforation. In still others, the network of the interstitial tissue extended up to the canal and the poorly-defined wall may have been due to a thickening of the network or to the presence of extremely fine particulate material. It was impossible to make an accurate judgment here, for the nature of the darkening was not resolvable, microscopically. It is interesting to note in these worms from down-river, that not all of the peripheral areas of the nephridial perforations were well-defined, but that all were surrounded by an area that stained darker with hematoxylin than the interstitial tissue. Indeed, in the case of those lumen perforations which were closed most tightly, the darkened areas helped to locate the fine canal openings.

As stated above, the down-stream form stands in contrast to its up-stream counterpart in the lesser amount of vascularization of the nephridium. Of the two nephridial halves referred to, the more lateral is the more vascularized (Fig. 6, BV). The nephridium of the down-river form is extremely well-supplied with a network of small blood vessels that ramify over its surface (especially that of the lateral half). These find their origin in the ventral segmental blood vessel, which itself proceeds over the anterior face of the medial half of the nephridium, and finally departs toward the parapodium, about 60 micra from the body wall. In the central part of the nephridium, this vessel gives rise to a branch that remains in contact with the medial half until immediately before ventral contact is made with the body wall (Fig. 5, VS). This last branch and the large ventral segmental vessel are the only blood vessels in contact with the medial half of the nephridium.

In the up-river form, the post-septal canal is fairly short and has a lumen diameter much the same as that of the main canal in the nephridial mass; in the down-river form (worms of comparable size) the post-septal canal is twice as long (about 250 micra) and the lumen is almost entirely closed at many points (Fig. 9B). The post-septal canal wall of the down-river form is also much thinner, about 1 to 2 micra for the most part. The small size of the tubule makes it difficult to trace from the nephridial mass to the nephrostome, for it is closely applied to the ventral segmental vessel throughout (Fig. 8), and at times, in transverse section, resembles

a small cell attached to the blood vessel. The nuclei which are visible within the tube wall possess little chromatin. The "septal band" separating the post-septal canal from the nephrostome is composed of a concentration of nuclei, but is not so extensive as the bands previously described for the up-river form and *N. vexillosa* (Fig. 8, NB).

The nephrostome (Fig. 8), which extends anteriorly about 100 micra from the dark band, has walls that appear to be solid, and there are no large vacuoles within them. The walls seem to be about the same density as the interstitial tissue of the nephridial mass. Around the margin of the nephrostome, the protoplasmic processes are stout, club-shaped structures that give rise to long cilia (Fig. 8, CP). Their shape would seem to bear out Goodrich's (1945) statement concerning the specificity of these structures, for they are similar to those observed in the up-river form, but differ from those of *Nereis vexillosa* and *N. diversicolor*. As before, the number of cilia originating inside the funnel is sufficient to clog the lumen (Fig. 8, CM).

Figure 11 shows the diameter of the nephridial lumen of the down-river form of *Nereis limnicola*. It is seen that the lumen is quite narrow at its beginning (of the order of 1 to 6 micra) and gradually increases in size, until at the three-quarter mark, it is consistently larger. Beyond this point, it undergoes a series of irregularities, grows extremely wide, closes once more, and finally becomes fairly uniform close to the nephridiopore. It is fully walled throughout; at, and just subsequent to, its widest part, the wall is at its thinnest; also, the wall thickens considerably as it approaches the nephridiopore. In the case of the nephridium upon which the diagram is based, the length of the canal within the nephridial mass is about 3864 micra, with a mean diameter of 9.3 micra.

The region of the nephridiopore of the down-river form of *N. limnicola* is essentially the same as that of the up-river form. As the nephridial canal approaches the body wall, the walls of the canal thicken, and contain large, relatively clear, nuclei. At times, the area shows the same compression as described for the up-river form.

Discussion

Several points emerge from the descriptions above: the nephridia of both the up-river and down-river forms of *N. limnicola* are more highly vascularized than those of *N. vexillosa;* the nephridia of the up-river form are more highly vascularized than those of the down-river form; the down-river form possesses a longer and more narrow nephridial canal than the specimens from up-river; and the nephridial blood vessels of both forms do not come into contact with the nephridial canal.

Krishnan (1952) found that the nephridia of *Namalycastis indica,* a euryhaline species, were larger and more heavily vascularized than those of the other nereids he studied. He also found that some of the nephridial blood vessels were in intimate contact with the canal wall and that, in the case of worms acclimatized to full-strength sea water, there was a lessening of the blood supply to the nephridia, in terms of shrunken and collapsed vessels. He suggested (p. 248) that the reduced blood supply might indicate that these nephridia ". . . are probably doing less osmotic work than in the normal forms living in fresh water." Krishnan fur-

ther postulated that there is a direct relationship between the size of nephridia and the osmoregulatory ability of the species in question, and that the ability of a nereid to osmoregulate also was reflected, not only by the amount of nephridial vascularization, but by the proximity of blood vessels to the nephridial canal.

It would seem from the series of three species considered by Krishnan that there is, indeed, a correlation between nephridial size and the ability to osmoregulate; but it should be noted that *Nereis vexillosa*, a stenohaline, relatively high-salinity species, possesses nephridia nearly as large as those of *Namalycastis indica* (Jones, 1957). Further, the nephridia of the up-river form of *Nereis limnicola*, which one would assume to be osmoregulating, are larger than those of *N. indica*, but quite a bit smaller than those of the down-river form which one would assume to be doing less osmoregulatory work.

TABLE I

Derivation of Indices of Excretory Capacity of nephridia from specimens of Nereis limnicola, from up-river (S-2), adapted from low to high salanity (S-1), from down-river (S-3), adapted from high to low salinity (S-4)

Worm	A Number of sections counted	B Assumed total number of canal sections (A × 3)	C Assumed length of canal (B × 6 μ)	D Number of segments	E Length (μ)	F Index of Excretory Capacity $\left(\frac{C \times 2D}{E}\right)$
S-2	128	384	2304	50	38,000	6.982
	100	300	1800	50	38,000	5.455
	98	294	1764	50	38,000	5.345
	86	258	1548	50	38,000	4.691
S-1	237	711	4266	42	30,000	11.945
	194	582	3492	42	30,000	9.778
S-3	290	870	5220	61	35,000	18.191
	274	822	4932	61	35,000	17.192
	224	672	4032	61	35,000	14.054
	252	756	4536	61	35,000	15.811
S-4	243	729	4374	62	33,000	16.436
	194	582	3492	62	33,000	13.121

Clearly, some character other than size, alone, allows these various nereids to survive in a dilute medium. Krishnamoorthi (1963b, 1963c) invoked size as a criterion of regulatory ability but, in addition, suggested that the length of the nephridial canal, as embodied in his "Index of Excretory Capacity" (= length of excretory surface, in microns/length of worm, in microns; "excretory surface" is defined as the average length of nephridial canal multiplied by the average number of nephridia per worm), was also a reflection of osmoregulation. Krishnamoorthi found that the indices of excretory capability were correlated with the distribution of four polychaetes, as he found them in the River Adyar and the nearby Bay of Bengal (Krishnamoorthi, 1963a): *Diopatra variabilis* Southern, index = 0.350, salinity range = 20–26‰; *Euclymene insecta* (Ehlers), 0.310, 20–26‰; *Onuphis eremita* Audoin and Milne Edwards, 0.247, 30–34‰; and *Loimia medusa* (Savigny), 0.225, 30–34‰. Although an extended series of pertinent observations

was not conducted on the length of nephridial canals of the up-river and down-river forms of *N. limnicola*, certain assumptions can be made. If one assumes that the number of canal sections counted in every third nephridial section (Table I, column A) is a reasonable estimate of one-third of the total number of canal sections, then, by multiplying by three (Table I, column B) and by the thickness of the sections, 6 microns (Table I, column C), one can arrive at an estimate of the length of a given nephridial canal. If this number is multiplied by twice the number of segments of the worm and this is, in turn, divided by the worm's length, in microns, one obtains Krishnamoorthi's Index of Excretory Capacity. Depending upon which nephridium and which population is chosen, the indices vary from 4.691–6.982 for the up-river forms to 14.054–18.195 for the down-river forms (Table I, column F). Worms cross-adapted from high to low and from low to high salinities give intermediate indices.

It would seem, intuitively, that the up-river population would have need of a greater "excretory capability," yet it has the lowest indices of the specimens of *N. limnicola* considered here. In addition, the lowest of the index values are more than ten times those found by Krishnamoorthi. Clearly, then, the Index of Excretory Capacity, itself, can not give an adequate idea of the osmoregulatory capabilities of a polychaete living in a low-salinity or fresh-water habitat.

Krishnan (1952) and Krishnamoorthi (1963b, 1963c) also have suggested that there is a correlation between the amount of vascularization and the ability to osmoregulate. Although subjective observations of the amount of nephridial vascularization of *N. limnicola* would seem to confirm this, I have not found a satisfactory method of quantifying these differences.

Yet another nephridial parameter might be considered, in addition to overall nephridial size, relative length of nephridial canal, and nephridial vascularization. Reduced to essentials, the survival of an animal with a permeable integument in a hyposmotic medium depends on (a) its ability to control its volume and, in effect, to slow or stop the osmotic inflow by hydrostatic pressure; (b) its ability to tolerate a dilution of its body fluids; or (c) its ability to counteract the dilutive effect of the osmotic inflow by the rapid excretion of water. Although the first two possibilities are outside the purview of the present work, observations have been made above, which bear on the third.

A number of papers have appeared which have been concerned with various physiological responses of *N. limnicola* to dilute or fresh water media. All of these postulate that there must be some means of volume control (Smith, 1963), a means of modifying salt loss rate (Smith, 1963), and/or a means of increasing the ability of the worm to eliminate excess water (Smith, 1959a, 1963; Oglesby, 1965b).

It has been noted that there is an apparent difference in the diameter of the nephridial canal of the two forms of *N. limnicola* considered here. In an effort to establish the statistical validity of these apparent differences, a number of nephridia of both forms were examined (Table II). Using 14 nephridia from six different up-river specimens and ten nephridia from four different specimens from the down-river area, all of the perforations of sectioned nephridial canals were measured in every third section of each nephridium. The results of all measurements of all nephridial canals of both forms were cast as frequency distributions (Fig. 12), and it was found that the mean canal diameter of the up-river forms

TABLE II

Collection data and various measurements of specimens of Nereis limnicola considered in the present study

Worm	Collection data		Salinity at death	Body segments	Length mm.	Mean canal diameter (μ)	Mean ± 2 S.E. (μ)
	Date	Salinity					
Nereis limnicola, Up-river							
S-2	10 Dec. 1950	0.55% SW	0.75% SW	50	33	22.84	21.28–24.40
S-2	10 Dec. 1950	0.55% SW	0.75% SW	50	33	16.82	15.64–18.00
S-2	10 Dec. 1950	0.55% SW	0.75% SW	50	33	11.03	10.03–12.03
S-2	10 Dec. 1950	0.55% SW	0.75% SW	50	33	10.66	9.66–11.66
RB	6 May 1951	2.45% SW	2.45% SW	52	—	30.36	28.18–32.54
RB	6 May 1951	2.45% SW	2.45% SW	52	—	21.59	19.63–23.55
S-10	6 May 1951	2.45% SW	2.45% SW	46	—	19.27	18.09–20.45
S-10	6 May 1951	2.45% SW	2.45% SW	46	—	17.74	16.60–18.88
49C	13 Jan. 1949	1.49% SW	1.49% SW	—	—	31.87	30.57–33.17
49C	13 Jan. 1949	1.49% SW	1.49% SW	—	—	27.26	25.08–29.44
49D	13 Jan. 1949	1.49% SW	1.49% SW	—	—	20.07	18.23–21.91
49D	13 Jan. 1949	1.49% SW	1.49% SW	—	—	18.51	16.27–20.75
49E	13 Jan. 1949	1.49% SW	1.49% SW	—	—	8.59	7.55– 9.63
49E	13 Jan. 1949	1.49% SW	1.49% SW	—	—	9.21	8.07–10.35
All data pooled	—	—	—	—	—	20.79	20.48–21.10
Nereis limnicola, Down-river							
S-3	10 Dec. 1950	81.00% SW	106.00% SW	61	35	9.79	8.97–10.61
S-3	10 Dec. 1950	81.00% SW	106.00% SW	61	35	9.54	8.72–10.36
S-3	10 Dec. 1950	81.00% SW	106.00% SW	61	35	9.09	8.37– 9.81
S-3	10 Dec. 1950	81.00% SW	106.00% SW	61	35	8.83	8.15– 9.51
SB	6 May 1951	48.00% SW	48.00% SW	64	—	4.83	4.23– 5.43
SB	6 May 1951	48.00% SW	48.00% SW	64	—	4.18	3.74– 4.62
S-13	6 May 1951	48.00% SW	48.00% SW	68	—	4.63	4.07– 5.19
S-13	6 May 1951	48.00% SW	48.00% SW	68	—	4.33	3.89– 4.77
51A	21 Feb. 1951	47.50% SW	47.50% SW	57	—	11.21	10.11–12.31
51A	21 Feb. 1951	47.50% SW	47.50% SW	57	—	10.88	9.64–12.12
All data pooled	—	—	—	—	—	8.49	8.19– 8.79
Nereis limnicola, Cross-adapted							
S-1	29 Apr. 1951	0.55% SW	118.00% SW	42	30	22.19	20.85–23.53
S-1	29 Apr. 1951	0.55% SW	118.00% SW	42	30	11.89	11.17–12.61
S-4	1 June 1951	81.00% SW	0.80% SW	62	33	17.32	16.40–18.24
S-4	1 June 1951	81.00% SW	0.80% SW	62	33	13.58	12.82–14.34
Nereis vexillosa, San Francisco Bay							
V1	3 May 1951	73–90% SW	73–90% SW	—	—	16.92	15.56–18.28
V1	3 May 1951	73–90% SW	73–90% SW	—	—	16.75	15.53–17.97
V2	3 May 1951	73–90% SW	73–90% SW	—	—	14.17	13.13–15.21
V2	3 May 1951	73–90% SW	73–90% SW	—	—	15.11	13.67–16.55
V4	3 May 1951	73–90% SW	73–90% SW	—	—	15.67	14.01–17.33
V4	3 May 1951	73–90% SW	73–90% SW	—	—	15.73	14.33–17.13

was 20.79 micra (one standard error = 0.31 micra) and that of the down-river forms was 8.49 micra (one standard error = 0.15 micra). Utilizing the "Student" t test, it was found that there was, indeed, a significant difference between the mean canal diameters of the two forms ($t = 38.44$). This also can be interpreted as the difference between the two means being 38.44 times the standard error of this difference.

The results above, however, may not be so straightforward as they might seem. If the mean canal diameter ($\pm$ 2 standard errors) of each nephridium examined is plotted against salinity (Fig. 13), it is seen that there is a rather large spread of the data derived from the up-river forms. Indeed, the results from three of the up-river worms (S-2, 49C and RB) indicate that there is a real difference between and among the diameters of the nephridial canal in the same animal, and

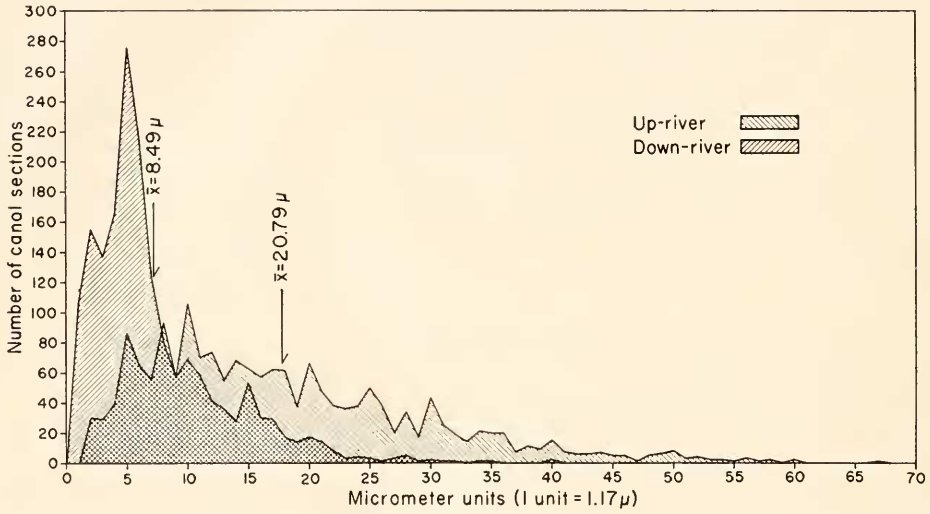


FIGURE 12. Frequency polygons showing the difference in nephridial canal diameter between the up-river forms of *Nereis limnicola* (based on 14 nephridia from six specimens) and the down-river forms (based on ten nephridia from four specimens).

two specimens (S-2 and 49E) have nephridial canals whose diameters are not significantly different from at least some of those from the down-river locality.

In addition to observations of the nephridia of worms sacrificed directly from the salinities in which they were collected, examinations were made of the nephridia of two cross-adapted worms. In the case of specimen S-1 (originally up-river), the adaptation was from 0.55% sea water to 118% sea water and of S-4 (originally down-river), from 81% sea water to 0.80% sea water. The general aspect of the nephridia of both S-1 (Figs. 14 and 15) and S-4 (Figs. 16 and 17) is strikingly similar to the nephridia of the up-river population of *Nereis limnicola*. The average canal diameter of S-4 (Fig. 13) falls among the lower values of the up-river forms, while that of S-1 is comparable to the larger canal diameters of the up-river population, even though S-1 was acclimatized to 118% sea water just before it was sacrificed.

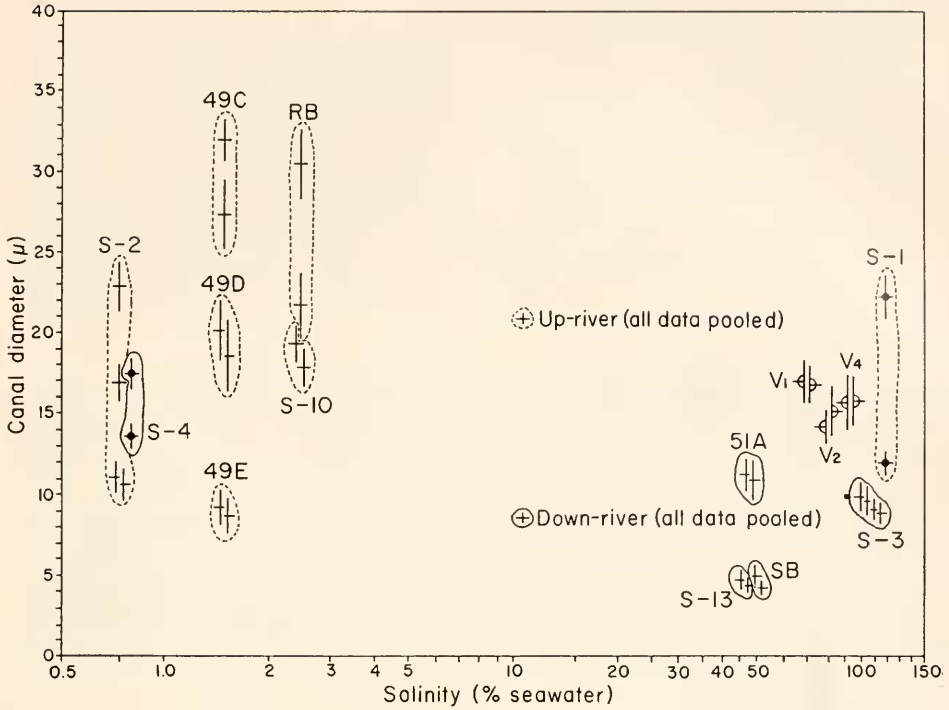
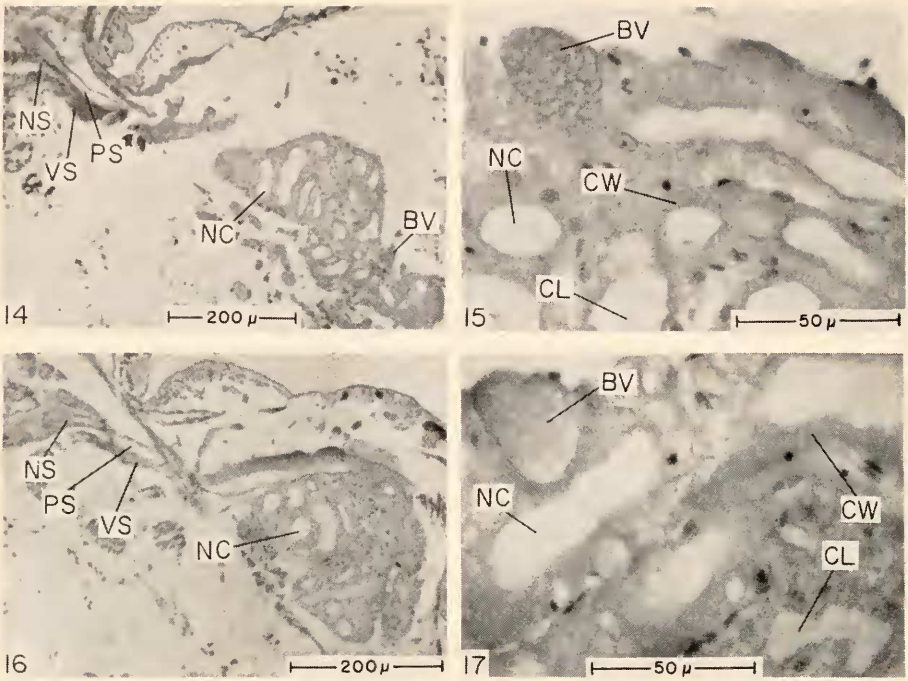


FIGURE 13. Graph showing the relationships among all nephridial canals considered. Horizontal lines represent mean canal diameters for each nephridium examined and the vertical lines, two standard errors above and below the mean. Specimen numbers are referable to Table II. Symbols surrounded by solid lines represent down-river forms; those with dashed lines, up-river forms; those with central solid circles, cross-adapted specimens; and those with central open half-circles, *Nereis vexillosa* from San Francisco Bay.

For comparison, Figure 13 also includes data based on observations of the nephridia of *Nereis vexillosa* from the Berkeley Yacht Harbor, San Francisco Bay (Jones, 1957). Although the salinity of the environment at the time of collection was not determined, the means ±2 S.E. for specimens V_1, V_2, and V_4 are clustered around an estimated salinity range, *i.e.*, 73–90% sea water (Jones, 1957, p. 407). The nephridial canals of *N. vexillosa* are significantly larger than those of the down-river forms of *N. limnicola* and are of comparable size to half of the up-river forms.

A comparison of the data of Table II indicates that, in the case of down-river forms, there is no difference between canal diameters of nephridia from the same segment (specimens S-3 and S-13) or from succeeding segments (specimen SB). In up-river forms, there is a significant difference in canal diameters of nephridia from the same segment in two of five cases (specimens S-2 and 49C), and in the one case of nephridia from succeeding segments (specimen RB). In both of the adapted specimens, S-1 and S-4, there is also a significant difference in the case of nephridia from the same segment.



Lettering as in Figures 1–8.

Figures 14 and 15, up-river form of *N. limnicola*, adapted from 0.55% sea water to 118% sea water; Figures 16 and 17, down-river form of *N. limnicola*, adapted from 81% sea water to 0.80% sea water.

FIGURE 14. Dorsal view of right nephridium and associated nephrostome; specimen S-1.

FIGURE 15. View of nephridial tissue; specimen S-1.

FIGURE 16. Dorsal view of right nephridium and associated nephrostome; specimen S-4.

FIGURE 17. View of nephridial tissue; specimen S-4.

Because of these apparently conflicting observations, that is, the small diameter of the nephridial canals of 49E and some of those of S-4 from up-river, and the large diameter of S-1, it is apparent that some physiological and/or physical mechanism, in addition to nephridial canal diameter, operates to allow *N. limnicola* to survive in dilute media.

That a larger canal diameter is advantageous in coping with lowered salinity is suggested by S-4 which apparently developed a larger nephridial canal as it was acclimatized from 81 to 0.80% sea water. That an environment of higher salinity does not necessarily evoke a comparable diminution of canal diameter is suggested by S-1 which apparently maintained a larger canal diameter in one of the measured nephridia while it was acclimatized from 0.55 to 118% sea water.

It would seem, then, that even though the annual fluctuations of salinity in the down-river area may be far greater than those up-river, nephridia with a relatively small diameter are adequate to the osmotic stresses placed on the worms in this area. On the other hand, the nephridia of the down-river forms appear to be more plastic in their response to a fresh-water or near-fresh-water medium; quite possibly, nephridial activity, insofar as water excretion is concerned, may be aug-

mented or superseded by some other mechanism. Finally, there appears to be a general trend toward nephridia of large lumen diameter in the up-river forms, although this is not invariably the case.

I would here extend thanks to Drs. Ralph I. Smith, Kenneth B. DeOme, and Howard A. Bern, all of the Department of Zoology, University of California (where much of the basic work of this study was carried out), to Dr. Larry C. Oglesby of Reed College, and to Drs. Marian H. Pettibone and Clyde Roper, Smithsonian Institution; the advice, criticisms, and suggestions of all of these have been gratefully received, if not always followed. I am particularly obliged to Dr. Smith for the use of a number of specimens of *N. limnicola* from his collections, both sectioned and un-sectioned.

Summary

1. The morphology of the nephridia of specimens of the polychaete worm, *Nereis limnicola* Johnson from areas of different salinity in the estuary of the Salinas River is described.

2. Generally, the canal diameters of the nephridia of the up-river (low salinity) forms are larger than those from down-river (high salinity); the nephridia of the up-river forms are more highly vascularized than those from animals found in higher salinities. This suggests that the nephridial canal acts to rid the animal of the excess water brought into its body by osmotic influx.

3. Nephridial canal diameters of worms adapted from low to high and from high to low salinities approach those of the animals from low salinity; this suggests that a larger canal diameter is efficacious in coping with the osmoregulatory problems presented by a dilute medium, and that canal diameter is not very important in higher salinities.

4. Inconsistencies in the correlation of large nephridial canal diameter with low salinity suggest that other mechanisms are utilized in meeting the stresses imposed by an environment of low salinity.

5. Krishnamoorthi's Index of Excretory Capacity is derived for a number of nephridia; the results indicate that the Index and/or the nephridia of *N. limnicola* do not seem to be comparable with Krishnamoorthi's observations on polychaetes of India.

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